

AD _____

Award Number: DAMD17-00-1-0203

TITLE: Training Program in Breast Cancer Research

PRINCIPAL INVESTIGATOR: Susan E. Kane, Ph.D.

CONTRACTING ORGANIZATION: City of Hope National Medical Center
Duarte, California 91010-3000

REPORT DATE: October 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030313 185

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

October 2002

3. REPORT TYPE AND DATES COVERED

Annual Summary (1 Sep 01 - 1 Sep 02)

4. TITLE AND SUBTITLE

Training Program in Breast Cancer Research

5. FUNDING NUMBERS

DAMD17-00-1-0203

6. AUTHOR(S):

Susan E. Kane, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)City of Hope National Medical Center
Duarte, California 91010-3000

E-Mail: skane@coh.org

**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE**13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)**

The objective of the City of Hope (COH) Breast Cancer Training Program is to develop a new generation of basic and clinical scientists trained to do research on breast cancer and its prevention. The Program will draw predoctoral trainees from the COH Graduate School and postdoctoral trainees from the basic sciences and clinical oncology disciplines at COH. Those who show a genuine interest in breast cancer research, as determined by a written application, are admitted into the Program. The overriding goal of the Breast Cancer Training Program is to provide outstanding training in the basic and translational science of cancer biology, with a special emphasis on breast cancer. Recognizing that cancer is a multi-faceted disease, trainees are required to work in laboratories that study the fundamentals of cancer biology and to develop research projects that focus on the special problem of breast cancer. Specific projects are approved by the Internal Advisory Committee, which includes experts in clinical research and basic science research, and leaders of the COH graduate school and clinical oncology training programs. In addition to completing their standard graduate and postdoctoral education, trainees in the Breast Cancer Training Program take inter-disciplinary coursework in 1) the biology and pathology of breast cancer; 2) breast cancer prevention and treatment; 3) the ethical conduct of basic and clinical research; 4) statistics as it relates to biological and cancer problems; 5) genetic pre-disposition to breast cancer and genetic counseling; and 6) quality of life/pain management of breast cancer patients. Trainees participate in seminars to share the results of their research and to review contemporary research literature in breast cancer; they attend lectures presented by speakers from outside of COH who are experts in the field of breast cancer; and they take advantage of a variety of seminars at COH having to do with other areas of basic science and translational research.

14. SUBJECT TERMS

training, inter-disciplinary, breast cancer biology and pathology, biostatistics/bioinformatics

15. NUMBER OF PAGES

16

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Key Accomplishments.....	4
Body.....	4-5
Reportable Outcomes.....	5-11
Conclusions.....	11
Appendices.....	12-16

PROGRESS REPORT

Title: Training Program in Breast Cancer Research

Number: DAMD17-00-1-0203

Principal Investigator: Susan E. Kane, Ph.D.

Key Accomplishments:

- Admitted 2 new trainees (1 graduate student, 1 research fellow) mid-year into the 2001-02 class and 4 new trainees (1 graduate student, 3 research fellows) into the 2002-03 class.
- Graduated 1 trainee (research fellow) from the program after two years. He remains at City of Hope as a senior scientist. One former trainee (research fellow) completed his postdoctoral training at City of Hope and now works at a pharmaceutical company in San Diego. Another trainee (graduate student) left City of Hope before completing her Ph.D. work and is currently working as a research technician at another institute. A spreadsheet listing the status of past and current trainees is attached as an appendix item.
- In the 2001-02 academic year, conducted formal courses in "Breast Cancer Biology and Pathology" and "Biostatistics and Bioinformatics." Provided course/lecture opportunities in the "Medical Oncology and Hematology Core Lecture Series" and "Topics in Cancer Genetics and Risk Assessment." Currently developing a mini-course in "Breast Cancer Clinical Trials -- Theory and Practice" for implementation in January, 2003 (see attached course outline).
- Continued holding bi-weekly meetings with all trainees and mentors alternating between Journal Club and Data Exchange formats, all centered on breast cancer research. Continued formal interactions (Journal Club, symposia, web discussions) with City of Hope's training program in Clinical Cancer Genetics. One of our trainees (clinical fellow) is now a joint fellow with the Clinical Cancer Genetics program.
- Supported high-quality research related to breast cancer biology (see summary of trainee activities below). Support for one trainee led to an application for an individual predoctoral fellowship from the DOD (pending).
- Sponsored or co-sponsored guest lectureships/seminars by breast cancer researchers, including Dr. Baibe Grube (John Wayne Cancer Institute), Dr. Judy Bolton (University of Illinois-Chicago), and Dr. Ellis Levin, (UC Irvine).
- Supported travel for 2 trainees to present their work at national meetings. In addition, 6 of our trainees (one supported on the institutional training grant, one with his own predoctoral fellowship from the DOD) attended and presented posters at the 2002 Era of Hope meeting. Two trainees were selected for oral presentations of their work.
- Recruited 2 additional faculty members as mentors -- Dr. Chih-Pin Liu (Assistant Professor of Immunology) and Dr. Rajesh Gaur (Assistant Professor of Molecular Biology).

Progress with respect to Statement of Work:

Task 1. Recruit trainees into the Breast Cancer Training Program. All items within this Task have been accomplished except for the development of advertising material to recruit new trainees, including minorities, to City of Hope (COH). Recruitment so far has taken place in the context of existing students and fellows at COH. Applications for admission into the program are solicited

annually, with review in August for admission into the program in September. We also conducted a mid-year solicitation and review this past academic year.

Task 2. Assign trainees to laboratories and identify breast cancer-related projects. This was accomplished for the 2000-01 and 2001-02 classes of trainees and has now been accomplished for the 2002-03 class.

Task 3. Initiate required coursework, journal clubs, and data exchange forums. Courses established in the first year of the program are still ongoing, as is the Journal Club and Data Exchange forum. In addition, we will be initiating a new course in "Breast Cancer Clinical Trials -- Theory and Practice" as of January, 2003 (see course outline in the Appendix).

Task 4. Monitor progress of past and current trainees and quality of the Program. Progress of graduate student trainees is monitored in the context of their required advisory committee meetings, which meet every 6-12 months for each trainee. Written progress reports are also received from all trainees as a requirement for their continuation in the program. The Internal Advisory Committee meets annually to review the program and to admit new trainees into the program (see minutes of meetings in the Appendix). A mid-year applicant review was conducted by e-mail. Trainee visibility is promoted by supporting travel to meetings.

Task 5. Establish a Distinguished Speaker series with experts in the field of breast cancer research. We continue to sponsor or co-sponsor several guest speakers each year. Meetings with our trainees are specifically arranged during the time these guests are on campus and trainees are required to attend the seminars. We will continue this practice and pursue the possibility of having an upcoming Beckman Symposium (an annual event attended by COH and other community scientists) be centered on gender-related cancers.

Specific trainee progress/Reportable outcomes:

Following are excerpts from trainee progress reports (those supported financially by the training grant in 2001-02) and a list of their specific accomplishments during the 2001-02 academic year. .

Carey Cullinane, M.D. (research fellow; mentors, J. Shively and B. Paz): (taken from meeting abstract) Annual mammography combined with physical examination is the current standard for early breast cancer detection. Current methods are far from perfect. Twenty to twenty-five percent of malignant lesions are not detected with screening mammography and fifty-five to eighty percent of patients who undergo biopsy based upon mammographic findings are diagnosed with benign disease. Carcinoembryonic antigen (CEA) is not produced in the normal breast epithelium but is produced in both pre-invasive and invasive disease. As the early abnormalities that progress to breast cancer first develop within the ductal system, utilizing secretions from the duct to detect CEA is an ideal adjunct to screening mammography. Methods: Five patients with a demonstrated mammographic abnormality scheduled to undergo biopsy or a definitive surgical procedure were enrolled in study. These included three patients with infiltrating ductal carcinoma (all with associated DCIS), one with papillary cystic carcinoma and one with proliferative breast disease. Nipple aspirate fluid (NAF) was collected from the breast with a mammographic abnormality in all patients and from the contralateral breast in two patients. Diluted NAF (1:50 and 1:100 dilutions in PBS) from all specimens was spotted on nitrocellulose membrane and dried. Purified CEA was spotted as a standard in triplicates in increasing amounts from 0.16ng to 160ng. CEA was detected using the monoclonal antibody T84.66 and a secondary horseradish-peroxidase labeled goat anti-mouse antibody, followed by chemiluminescence detection and 2 min exposure on film. Filters were then stripped and a similar process used to detect the presence of human serum albumin (HSA). Expression of CEA in all tumors was assessed by

immunohistochemistry utilizing the same monoclonal antibody. Results: NAF from the affected breast in all three patients with invasive ductal carcinoma was positive for both the presence of CEA and HSA. NAF from the patient with invasive papillary carcinoma was negative for both CEA and HSA. NAF from the patient with proliferative breast disease was negative for the presence of CEA but positive for the presence of HSA. The contralateral mammographically normal breast was negative for CEA but positive for HSA in the two patients in whom it was collected. Both invasive and non-invasive tumors demonstrated the presence of CEA by immunohistochemistry.

Abstract:

2002 Era of Hope - Department of Defense Breast Cancer Research Program Meeting (poster)
"Carcinoembryonic Antigen in nipple aspirate fluid as a marker of malignancy in women with mammographically detected abnormalities"

Toru Itoh, Ph.D. (research fellow; mentor, Shiuan Chen): (taken from meeting abstract) Using the yeast one-hybrid screening approach with a human breast tissue hybrid cDNA library, Snail and Slug zinc-finger proteins were identified to interact with the CREar element (cAMP-responsive element) in the promoter I.3 region of the human aromatase gene. These proteins have been shown as transcriptional repressors in many animal species including human, involved in mesoderm differentiation, epithelial-mesenchymal transition, brachial development and branching, neural crest cell migration, apoptosis and E-cadherin expression. Aromatase is an estrogen synthetase, and estrogen plays a critical role in breast cancer progression. This enzyme is expressed at higher levels in breast cancer tissues and cell lines than normal tissues, and its expression is driven by promoter I.3 in breast tumors whereas normal tissues generally use promoter I.4. DNA mobility shift assays and mutation analyses using recombinant Slug protein expressed in *E coli* have revealed that Slug protein interacts with CREar region whereas Snail protein binds to a segment, 5'-CTGATGAAGT-3', which is between 66 and 76 bp upstream from the transcriptional start site of promoter I.3. Using mammalian cell transfection experiments, Snail and Slug were found to act as repressors of promoter I.3 activity. Site-directed mutagenesis experiments have revealed that the N-terminal SNAG domain is important for the repressor activity of Snail. To demonstrate the inhibitory activity against aromatase expression, a stable Snail expressing MDA-MB-231 breast cancer cell line was generated, and the aromatase RNA messages in the Snail transfected cell line were found to be thirty percent of those in the vector transfected cell line. Furthermore, in order to better understand the functions of these two zinc-finger proteins in human breast tissue, we also stably transfected Snail, Slug and their antisense cDNAs in MCF7 (breast cancer cell line) and MCF10A (non-cancer breast epithelial cell line), respectively. These transfected cell lines were examined using three-dimensional culture in Matrigel. While MCF10A cells formed acinar structures containing a single layer of polarized growth-arrested cells in Matrigel, MCF10A cells transfected with the Slug antisense plasmid lost the ability to form the acini-like structure. MCF7 cells are Snail negative and Slug negative, and form disorganized structures in Matrigel. Interestingly, Snail or Slug over-expressing MCF7 cells form acini-like structure with lumen. These findings strongly suggest that Snail and Slug proteins are involved in cell polarization and acini formation. *In situ* hybridization and immunohistochemistry are being carried out to investigate the localization of mRNA and protein markers that are specific for cell polarization and lumen formation.

Abstract:

2002 Era of Hope - Department of Defense Breast Cancer Research Program Meeting (poster)
"Functional Characterization of Snail and Slug Zinc-Finger Proteins in Breast Tissue"

Julia Kirshner (graduate student; Jack Shively, mentor): (extracted from trainee's annual progress report) A model of mammary morphogenesis involves growth of mammary epithelial cells in a three dimensional culture of Matrigel (a source of extracellular matrix). The resulting acini include lumens which are thought to be formed by apoptosis of the cells within the center of the spherical colonies. Recent studies have shown that biliary glycoprotein (CEACAM1) which is a cell-cell adhesion molecule that is expressed on the luminal surface of most epithelial cells may be involved in lumen formation. Transfecting MCF7 cells with CEACAM1-4S restores lumen formation in Matrigel in these otherwise solid colony-forming cells. However, MCF7 cells transfected with CEACAM1-4L do not survive long enough in Matrigel for any meaningful assessment of lumen formation. With these results the following specific aims were formulated:

1. Determine the specific role of CEACAM1-4S in lumen formation.
 - a. Analyze the time frame for apoptosis in our model system.
 - b. Establish the biochemical evidence for apoptosis utilizing assays for cytochrome c extrusion, caspase activation nuclease activity (TUNEL), and PARP cleavage.
2. Determine the apoptosis signal transduction pathway, which CEACAM1 employs. Direct vs. indirect signaling. Does CEACAM1 directly trigger apoptosis or is it an intermediate in the signaling pathway? For example, does the cell-cell adhesion properties of CEACAM1 trigger gene expression of other apoptotic inducers.

Parts a and b of the specific aim 1 have been completed and progress has been made on aim 2. Taken together results demonstrate that the luminal localization of CEACAM1 is favorable to directly mediate apoptotic signaling resulting in the formation of the lumen. Furthermore, correct phosphorylation of CEACAM1-4S is crucial for both cell survival and cell death leading us to hypothesize that CEACAM1-4S is differentially phosphorylated on Thr and Ser residues in living and dying cells.

Manuscripts in preparation:

1. Julia Kirshner, Chang-Jui Chen, Ping-Fang Liu, Jie Huang, and John E. Shively (2002). The Short Cytoplasmic Domain of CEACAM1 Induces Lumen Formation by Apoptosis IN MCF-7 Mammary Carcinoma Cells.
2. Julia Kirshner, John D. Hardy, and John E. Shively (2002). Ultrastructural Studies of CEACAM1-4S Behavior During Lumen Formation in MCF7 Cells.

Abstracts:

1. Research Staff Organization Advance (poster)
"Phosphorylation of CEACAM1-4S Induces Lumen Formation and Apoptosis in Transfected MCF-7 Mammary Carcinoma Cells"
2. 2002 AACR Annual Meeting (poster)
"Phosphorylation of CEACAM1-4S Induces Lumen Formation and Apoptosis in Transfected MCF-7 Mammary Carcinoma Cells"
3. 13th Annual International CEA Symposium (talk)
"Phosphorylation of CEACAM1-4S Induces Lumen Formation and Apoptosis in Transfected MCF-7 Mammary Carcinoma Cells"
4. 2002 Era of Hope - Department of Defense Breast Cancer Research Program Meeting (poster/talk)
"Phosphorylation of CEACAM1-4S Induces Lumen Formation by Apoptosis in Transfected MCF-7 Mammary Carcinoma Cells"

Chunxia Li (graduate student; mentor, Ren-Jian Lin): (extracted from trainee's annual progress report) Breast cancer is a heterogeneous disease which stems from genetic and environmental factors

resulting in the accumulation of mutations in essential genes including BRCA1, BRCA2, P53, RB (retinoblastoma gene), ErB2, *c-myc* and cyclin D1. On the other hand, growing evidence indicates that alternative or aberrant pre-mRNA splicing takes place during the development, progression, and maintenance of breast cancer. These genes include receptor proteins (estrogen receptors and prolactin receptors), tumor suppressor genes (*ZAC* and *TSG101*), and breast cancer susceptibility genes (*BRCA-1* and *BRCA-2*), CD44, fibroblast and vascular endothelial growth factor receptors, and Her2/neu. However, it is very likely that additional alternative splicing events are occurring in breast cancer. This assumption is supported by a recent study showing that SR proteins, a family of splicing factors that are often involved in binding to splicing enhancer sequences, undergo stage-specific changes in mouse mammary tumorigenesis. However, it is not clear whether these splice variants are linked or contribute to human breast cancer. In this project, I am developing and using microarray techniques to simultaneously assay splicing changes of a number of genes in a variety of breast cancer cell lines. The microarrays and cell lines should allow me to determine the correlation between alternative splicing changes and breast cancer development. I also propose to use small interference RNA (siRNA) to specifically inactivate the splice variant of interest in order to clarify the importance of that splice variant in breast cancer. I believe these studies will provide potential use in diagnosis or treatment for breast cancer.

Results to date: 1) I have annotated alternative splicing patterns of 35 breast cancer related genes reported in all of the literature. 2) Since we are going to use oligonucleotide microarray technique to detect the splice variants of our genes of interest, I am designing specific oligonucleotides for each gene. The oligos I am designing are 40mer sense oligos which are complimentary to the exon-exon junctions. I am also including some oligos complimentary to the constitutive exons which can serve as internal controls. 3) Initially, we obtained microarray slides from UC Santa Cruz that contain >400 oligonucleotides designed to detect alternatively spliced mRNAs from more than 60 human genes implicated in cancer progression and apoptosis. Poly(A)⁺ RNA was isolated from human mammary epithelial cells and MCF7 breast cancer cells, labeled with Cy3 or Cy5, and hybridized to the glass oligonucleotide arrays. We are currently optimizing conditions for hybridizations and probe preparation. Future work will focus on 1) Designing and printing our own microarray slides; 2) testing breast cancer cell lines including MCF-7, T47D, MDA-MB-231, and BT-474 for alternative splicing patterns in candidate genes; 3) testing clinical samples for alternative splicing patterns; 4) using small interference RNA (siRNA) to specifically inactivate a splice variant of interest in order to clarify the importance of that splice variant in breast cancer.

Abstract:

2002 Era of Hope - Department of Defense Breast Cancer Research Program Meeting (poster)
 "Investigation of Alterations in Pre-mRNA Splicing in Breast Cancer Using Oligonucleotide Microarrays"

Grant application:

Predoctoral fellowship application to DOD 2002 Breast Cancer Research Program.

"Investigation of Alterations in Pre-mRNA Splicing Breast Cancer Using Oligonucleotide Microarrays and Small Interference RNA" #BC021575

Tove Olafsen, Ph.D. (research fellow; mentor, Anna Wu): (taken from meeting abstract)

HER2/neu is overexpressed in ~30% of human breast and ovarian cancers. The relatively low expression of this antigen on normal tissues makes this an attractive target. Monoclonal antibodies (mAbs) can be used for delivery of radioisotopes for imaging and therapy. For imaging, intact mAbs (150 kDa) are not suitable as they clear too slowly from the circulation. On the other hand, engineered single-chain Fv (scFv, 27 kDa), consisting only of the variable (V) domains, clears too fast.

Intermediate fragments such as minibodies (single-chain Fv-CH₃ fusion proteins, 80 kDa) against carcinoembryonic antigen (CEA) on colon cancer cells have been shown to exhibit rapid, high tumor uptake in xenografts. Moreover, this minibody, labeled with the positron emitting radionuclide, ⁶⁴Cu (t_{1/2} = 12.7 h), has shown high-resolution microPET images of xenografts in mice. These results led us to generate and evaluate minibodies against a new target, HER2/neu. In this study we used V genes from two different anti-HER2/neu mAbs, 10H8 [Park J.M. *et al.* Hybridoma 1999, 18:487] and Herceptin. The V genes were assembled into scFv fragments and joined to the third constant domain of human IgG1 (CH₃γ₁). Constructs were expressed in the mouse myeloma cell line, NS0, at 20-60 μg/ml. Binding to the overexpressing breast cancer cell line, MCF-7/Her, was shown by flow cytometry. Furthermore, the two different minibodies were shown to bind to different epitopes on the antigen by competition. Proteins were purified to >95% purity. Purified protein was conjugated with the chelating agent DOTA and labeled with ¹¹¹In for biodistribution studies. The 10H8 minibody showed low to moderate tumor uptake and high kidney uptake relatively to the anti-CEA minibody. The Herceptin minibody, evaluated in non-tumor bearing mice, showed similar uptake in the kidneys. This was unexpected, as the size of the minibody is larger than that for the renal filtration threshold (<60 kDa). The Herceptin minibody was also labeled with ⁶⁴Cu and evaluated in xenografts using a microPET scanner. Again, tumor uptake was low relative to the kidney and liver. Immunohistochemical staining of normal mouse kidneys showed strong specific staining of the proximal tubules. We conclude that the minibodies may be filtered via the kidneys and that they recognize and bind to an antigen in the proximal tubules. Hence, use of anti-HER2/neu fragments for imaging will require further engineering and development.

Abstract:

2002 Era of Hope - Department of Defense Breast Cancer Research Program Meeting (poster/talk)
"Evaluating two different minibodies, (scFv-CH₃)₂, against HER2/neu for imaging of breast cancer"

Corinne Solier, Ph.D. (research fellow; mentor, Barry Forman): (taken from meeting abstract)
Ecteinascidin-743 (ET-743), a novel natural marine molecule currently undergoing Phase II clinical trials, is an extremely potent anticancer drug acting on a broad panel of tumors, including soft tissue sarcoma, which mechanisms of action still remain unclear. A structural analog of ET-743, phthalascidin (PT-650), which displays similar antiproliferative properties as ET-743, has recently been synthesized. Our study aims at identifying the specific cellular targets of ET-743 and PT-650, mediating their antineoplastic and antiproliferative effects. Such a study will provide new tools for the future screening of more effective analogs in order to develop alternate chemotherapeutic treatments. In the current work, we showed that ET-743 and PT-650 display transcriptional inhibitory properties on a broad but yet specific panel of nuclear hormone receptors, using transfection-based reporter assays in CV-1 cells. More specifically, we showed that ET-743 inhibits the ligand-induced transactivation of those receptors without affecting their basal transcriptional activity. To delineate the molecular mechanisms of action supporting those transcriptional inhibitory effects, we first performed *in vitro* Electrophoretic mobility Shift Assays (EMSA), which indicated that the transcriptional inhibitory effects of PT-650 are indirect. This result suggests the requirement for the recruitment of a mediator protein on the receptors by PT-650 in order to exert its transcriptional inhibitory properties. Mammalian two-hybrid assays further evidenced the displacement of co-activator molecules from the ligand-bound nuclear receptors specifically targeted by PT-650. Altogether, those data demonstrate that ET-743 and PT-650 inhibit the ligand-induced transcriptional activity of nuclear hormone receptors in a broadly specific manner, which may account for the broad cytotoxicity of those molecules on tumors. As suggested by EMSA and two-hybrid assays performed using PT-650, the mechanism mediating the transcriptional inhibitory properties of those compounds may involve the

recruitment of a cellular protein on the targeted nuclear receptors, which would then lead to a displacement of co-activator factors from the receptors.

Manuscript:

Lamorte, V.J., Krasieva, T., Zoumi, A., Forman, B.M. and Solier, C. One-photon versus two-photon excited fluorescence resonance energy transfer of GFP fusion proteins. *Proc. Soc. Photo-opt. Instrum. Eng. In press.*

Abstract:

2002 Orphan and Nuclear Receptors, San Diego, CA (organized and sponsored by the Knowledge Foundation). "Inhibition of nuclear receptors by the anticancer drug ET-743"

Xiangrong Zhao (graduate student; mentor, Chih-Pin Liu): (extracted from trainee's annual progress report) The genesis of breast cancer can be viewed as the result of the accumulation of damage over many years to cells within the breast, including mutations of DNA and loss of control of cell proliferation. The lack of apoptosis plays a significant role in the etiology of cancers. Human LUCA-15 maps to 3p21.3, a region of the chromosome important to a number of cancers, including lung and breast cancers. There is a high (over 90%) correlation between the loss of heterozygosity (LOH) at 3p21.3 and several types of cancer, implying the existence of a tumor suppressor gene in this region. The localization of human LUCA-15 to the 3p21.3 homozygous deletion region indicates that it may be a tumor suppressor gene. However, interestingly, in both primary cells and cell lines from breast cancers, a direct correlation has been shown between up-regulation of LUCA-15 RNA (Clone H37) and HER-2/neu oncogene overexpression, suggesting that human LUCA-15 might in fact be an oncogene. Overexpression of LUCA-15 itself suppresses CD95-mediated apoptosis in Jurkat T cells. This suppression occurs prior to the final execution stage of the CD95 signalling pathway, and is associated with up-regulation of the apoptosis inhibitory protein Bcl-2. LUCA-15 was demonstrated to be a selective inhibitor of cell death. Moreover, overexpression of an alternative RNA splice variant of LUCA-15 has been shown to retard human Jurkat T cell proliferation and to accelerate CD95-mediated apoptosis. An antisense cDNA to the 3'-UTR of this splice variant was able to suppress CD95-mediated apoptosis.

Little has been known about the exact mechanism underlying the potentially dual roles of LUCA-15 as an apoptosis inhibitor and inducer, the possible interplay among its splicing variants, and the sequential effects in carcinogenesis. To take advantage of the comparable easy-accessibility and versatility of mouse models, I proposed to use mouse LUCA-15 homologue for in vitro biochemistry assays and the further creation of transgenic mice, to study the possible roles of this evolutionarily conserved molecule in the general cell growth control and in the carcinogenesis/progression in breast cancer. Our results to date indicate that: 1) There is a mouse homologue of human LUCA15. The mouse LUCA15 ORF is 2,445 bp, with 92% homology in nucleotide sequence to human LUCA15. 2) Mouse LUCA15 encodes a full-length mRNA and at least another 4 natural splicing variants, as determined by sequencing of RT-PCR products. 3) I have engineered plasmid constructs with either full length (wild type) or the artificially truncated (splicing variants) mouse LUCA15 cDNAs in a pFLAG retroviral vector. The expressed fusion proteins have N-terminal mouse LUCA15, an internal FLAG tag for IP and Western blotting, and C-terminal GFP for screening and cellular distribution studies. 4) I have obtained positive transfectants of all the fusion proteins of truncated mouse LUCA15 and the empty pFLAG control, according to the GFP fluorescence observed under UV. No green fluorescence has been detected yet with the full length mouse LUCA15 transfectants although after G418 selection some cells are still alive. Further Western blot will determine the efficiency of transfection, expression, and post-translational folding. 5) RT-PCR analysis has indicated that mouse LUCA15 is ubiquitously expressed in mouse organs/tissue types; mouse LUCA15 expression is

possibly related to T cell development status; and different splicing variants may have different effects on T cell development status.

Additional Reportable Outcomes (past trainees):

Manuscripts:

1. Negritto, M. C., J. Qiu, **D. O. Ratay**, B. Shen, A. M. Bailis. 2001. A novel function of Rad27/FEN-1 in restricting short-sequence recombination. *Mol. Cell. Biol.* 21:2349-2358.
2. Dussault, I, **H-D Yoo**, Min Lin, AK Batta, G Salen, S Erickson and BM Forman (2002) Endogenous ligands for mouse but not human PXR accumulate in CYP27 deficiency and Cerebrotendinous Xanthomatosis. *Submitted.*
3. Dussault, I, **H-D Yoo**, M Fan, J Hou, IP Schulman, and BM Forman (2002) Endogenous and synthetic antagonists of the nuclear receptor LXR inhibit lipogenesis. *In preparation.*

Abstracts:

Negritto, M.C., J. Qiu, **D.O. Ratay**, B. Shen, and A.M. Bailis. 2001 A role for Rad27/FEN-1 in restricting short-sequence recombination. Gordon Conference on Radiation Oncology, Ventura, CA

Conclusion:

We have accomplished most of goals for the Training Program in Breast Cancer Research at City of Hope. Since inception, we have fully or partially supported 6 graduate students, 8 postdoctoral fellows and 1 clinical oncology fellow. This continues to exceed our expectations in the original training grant application. All of these trainees are working on breast cancer-related projects and all are attending a variety of breast cancer-related courses, seminars, journal clubs, and data sessions. Importantly, our trainees have been highly competitive for obtaining their own independent fellowships, based largely on the training environment that we can provide in our breast cancer research program. We have had outstanding participation by the faculty mentors of these trainees as well. Notably, we have recruited several mentors (through their trainees) who have not traditionally worked in the field of breast cancer research, thus expanding their scope of research and adding fresh perspective to our trainee's experience. We are continuing to add interesting coursework that draws on the clinical environment at City of Hope, with outstanding participation from the institution's clinical staff.

APPENDIX 1 -- New Course Outline

Breast Cancer Clinical Trials -- Theory and Practice

5:30 - 7:30pm

January 8	Basic Principles of Clinical Trials	Lucille Leong
January 15	Breast Cancer Clinical Trials at COH	Lucille Leong Protocol PI's
January 22	Translational Research: From the Laboratory to the Clinic	James Doroshow
January 29	Clinical Correlates: From the Clinic to the Laboratory	Ned Newman
February 5	Procurement and Processing of Human Specimens; The Analytic Pharmacology Core	Tim Synold
February, TBD	Clinic Module -- Visits to Patients Enrolled on Clinical Trials	Lucille Leong

Students will also be provided with the book "Protecting Study Volunteers in Research" and will take the Protecting Study Volunteers Examination required of all COH investigators participating in clinical and translational research.

APPENDIX 2 -- Journal/Data Club Schedule

BREAST CANCER JOURNAL/DATA CLUB SCHEDULE -- 2002-03

October 1	Data	Tove Olafsen
October 15	Journal	Carmel Chan
November 5	Data	Julia Kirshner
November 19	Journal	Corinne Solier
December 3	Data	Chunxia Li
December 17	Journal	Xiangrong Zhao
January 7	Data	Jennifer Murray
January 21	Journal	Sethu Nair
February 4	Data	Detlef Schumann
February 18	Journal	Damon Meyer
March 4	Data	Donna Brown
March 18	Journal	Mitsuo Kato
April 1	Data	Carey Cullinane
April 15	Journal	Tove Olafsen
May 6	Data	Carmel Chan
May 20	Journal	Julia Kirshner
June 3	Data	Corinne Solier
June 17	Journal	Chunxia Li
July 1	Data	Xiangrong Zhao
July 15	Journal	Jennifer Murray
August 5	Data	Sethu Nair
August 19	Journal	Detlef Schumann
September 2	Data	Damon Meyer
September 16	Journal	Donna Brown
September 30	Data	Mitsuo Kato
October 7	Journal	Carey Cullinane

APPENDIX 3 -- Internal Advisory Committee Meeting Minutes

Breast Cancer Training Program Internal Advisory Committee Minutes for August 21, 2002

Present: S. Chen, S. Kane, S. Novak, J. Rossi, J. Weitzel, S. Wilczynski, Y.-C. Yuan
Applicant review sheets received by mail from: M. Grant, L. Wagman

The meeting was convened by S. Kane at 11:00am.

1. Dr. Kane presented a brief update on the progress of each trainee and the plans to support travel to the Era of Hope meeting in September, 2002. She also informed the group that a graduate student not supported by the training program had applied for and received a predoctoral fellowship from the California Breast Cancer Research Program. This student clearly benefitted from having the existing training environment offered by our program and she will now be a full participant in the training program with her own funding. There was also a review of the funds committed to existing trainees and funds available for admitting new trainees into the program (see item #3)
2. Each committee member had received trainee applications and applicant rating sheets (attached) for each one. The sheet uses an NIH-format rating system to score each application in the areas of Candidate, Project, Mentor and Overall. Committee member scores (excluding conflicts) were collected prior to and at the meeting and collated on a spreadsheet. Candidates were ranked according to scores and then there was discussion of the candidates and the rankings.
3. We had 6 applications all together, with funding designated for 1 graduate student and 1.5 postdoctoral fellows. One additional postdoctoral fellow was admitted in a previous round of applicant reviews but will be starting in the program in September, 2002. Results of the scoring were such that 1 graduate student (Damon Meyer) and 1 postdoctoral fellow (Detlef Schumann) were judged as superior candidates, an additional candidate (Donna Brown) was judged as outstanding and a third postdoc candidate (Mitsuo Kato) received excellent marks. It was agreed that Damon Meyer and Detlef Schumann would be supported with full stipends, Donna Brown would receive half-stipend support and Mitsuo Kato would be invited to participate in the program with stipend support pending future availability of training grant monies.
4. There was an update on continuing courses and discussion of a new course being planned for Winter, 2003 ("Breast Cancer Clinical Trials"). It was agreed that this was a good idea, including the plan to include visits to patients enrolled on trials and a requirement to read the "Protecting Study Volunteers in Research" book and pass the Protecting Study Volunteers Examination required of all COH investigators participating in clinical and translational research.
5. There was a brief discussion of early-stage plans to convert the DOD's Institutional Training grant into an NIH-funded T32 training grant.

Meeting was adjourned at 12:00pm.

Candidate's Name _____

APPLICANT RATING SHEET

Rate each element on a scale of 1-5, in increments of 0.1, with 1 being the highest.

Candidate _____

(qualifications; motivation; stage in career; potential as future breast cancer researcher)

Project _____

(relevance to breast cancer; appropriateness as a training tool)

Support from Mentor _____

(letter of recommendation; training environment in his/her lab; support for participation in training program)

Overall _____

Comments:

APPENDIX 4 -- List and Tracking of Trainees

Trainee	Years	Mentor	Status in Program	Current Employment	Current Position
Hye-Dong Yoo	2000-01	B. Forman	Postdoc	Sequoia Sciences, San Diego	Research Scientist
Dawn Ratay	2000-01	A. Bailis	Grad Student	Agensys, Inc., Santa Monica	Research Technician
Toru Itoh	2000-02	S. Chen	Postdoc	BRI/COH	Research Fellow
Carmel Chan*	2000-03	S. Kane	Grad Student		
Julia Kirshner	2000-03	J. Shively	Grad Student		
Tove Olafsen	2001-02	A. Wu	Postdoc	UCLA	Postdoctoral Fellow
Carey Cullinane	2001-03	B. Paz/J. Shively	Surgical Oncology Fellow**		
Chunxia Li	2001-04	R.J. Lin	Grad Student		
Corinne Solier	2002-04	B. Forman	Postdoc		
Xiangrong Zhao	2002-04	C.-P. Liu	Grad Student		
Sethu Nair	2002-04	R. Gaur	Postdoc		
Detlef Schumann	2002-04	J. Shively	Postdoc		
Donna Brown	2002-04	S. Kane	Postdoc		
Mitsuo Kato	2002-04	R.J. Lin	Postdoc		
Damon Meyer	2002-04	A. Bailis	Grad Student		

*Received Predoctoral Training Grant, DOD

**Joint Participation in Surgical Oncology, Breast Cancer and Clinical Cancer Genetics Fellowship Programs